Bdellovibrio and Like Organisms are Predictors of Microbiome Diversity in distinct Host Groups

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Abstract

Biodiversity is generally believed to be a main determinant of ecosystem functioning. This principle also applies to the microbiome and could consequently contribute to host health. According to ecological theory, communities are shaped by top predators whose direct and indirect interactions with community members cause stability and diversity. *Bdellovibrio* and like organisms (BALOs) are a neglected group of predatory bacteria that feed on Gram-negative bacteria and can thereby influence microbiome composition. We asked whether BALOs can predict biodiversity levels in microbiomes from distinct host groups and environments. We demonstrate that genetic signatures of BALOs are commonly found within the 16S rRNA reads from diverse host taxa. In many cases, their presence, abundance, and especially richness are positively correlated with overall microbiome diversity. Our findings suggest that BALOs can act as drivers of microbial alpha-diversity and should therefore be considered as candidates for the restoration of microbiomes and the prevention of dysbiosis.

Biodiversity is a key attribute of productive [1] and stable ecosystems [2]. This is likely due to the activity of highly productive keystone species [3], which are often more common in species-rich communities [1]. Nevertheless, productivity and stability appear to be mainly driven by diversity itself and not by individual taxa [4]. Species-rich communities exist for example in the human gut and oral microbiome and are usually assumed to consist of functionally redundant species that act as insurance in case of extinctions [5, 6]. Consequently, species-rich communities are more resilient (cf. [7]). To date, most studies on the effect of biodiversity on ecosystem functioning and specifically the effect of microbiome composition on host health have focused on a single trophic level. Yet, changes in the diversity of one trophic level can affect other trophic levels, either directly through consumer-resource interactions or indirectly when the decrease of one species leads to abundance changes of other species [8]. The presence of top predators has particularly strong effects because they can limit dominant species abundance and thereby free niches for rare taxa [9–11]. The impact of predators is likely distinct from environmental stressors, which may similarly free niches and subsequently increase microbiome diversity, as recently documented for the microbiome of *Daphnia* waterfleas after antibiotic exposure [12]. Yet, in this case, the effect on community composition is likely to be random, whereas predators usually target the dominant species.

Bdellovibrio and like organisms (BALOs) are obligate predators of Gram-negative bacteria in a wide range of habitats [13, 14]. BALOs were recently linked to a healthy human gut microbiome [15], and proposed as living antibiotics in medical treatment [16] and water remediation [17]. Additionally, a microcosm experiment showed that their predatory activity can exceed phage-induced mortality [18]. We here draw attention to this neglected group of predators and tested their association with microbial diversity as an indicator of a healthy microbiome across distinct animal host groups and environments.

We analyzed 16S rRNA data from randomly chosen, exemplary host taxa that are representative of distinct animal taxonomic groups, including early branching metazoans, ecdysozoa, selected vertebrates, and additionally home surfaces (Table S1 and Supplementary Methods). We only considered studies, if they included samples with and without BALOs, thereby allowing us to determine the consequences of BALO presence and absence in comparable groups. We determined BALO occurrence (although not necessarily activity) by identifying OTUs that showed 97% sequence identity to members of the BALO-containing taxonomic groups *Bdellovibrionales* (including the families *Bacteriovoracaceae* and *Bdellovibrionaceae)* and *Micavibrionales* (including *Micavibrionaceae)*. From these data, we inferred relative BALO abundance and corresponding microbiome alpha- (i.e., Shannon-Wiener diversity, Simpson's diversity, richness) and beta-diversities.

The presence of BALOs was associated with a significantly higher Simpson and Shannon diversity for the microbiomes of seven and five host species, respectively, as well as the home surfaces (Figure 1, Table S2). The main exceptions referred to two sponge species, *Carteriospongia foliascens* and *Ircinia variabilis*, which showed a significantly higher alpha-diversity in the absence of BALOs. This negative association was not observed for microbiome richness (Figure S1, Table S3). Our subsequent analysis of absolute OTU numbers revealed that microbiome richness is significantly associated with both BALO abundance (Figure 2a, Table S4) and BALO richness (Figure 2b, Table S4) in case of *H. vulgaris* and the sponges. A trend toward this association was additionally observed for *N. vectensis* and *D. melanogaster*.

Interestingly, for both host systems, OTU richness was highest with medium BALO abundance, which possibly indicates that BALO richness rather than abundance influences microbiome richness.

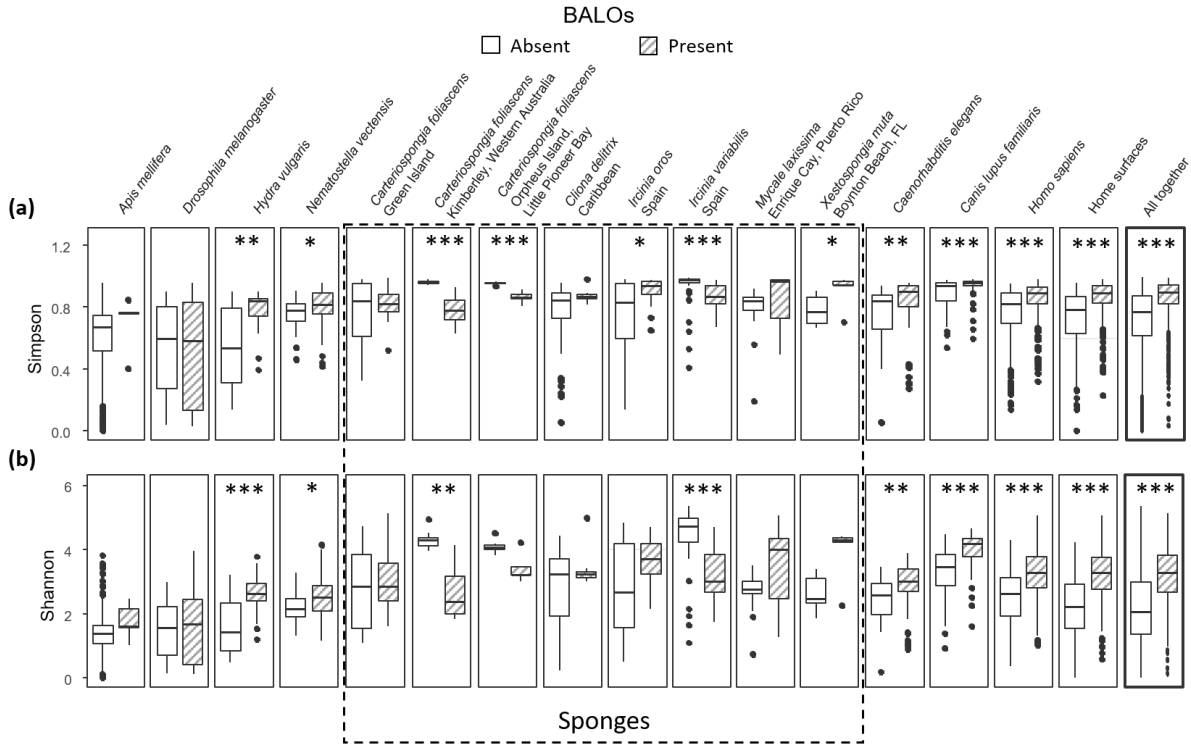

Fig. 1. Microbiome alpha-diversity in the presence and absence of BALOs. The Simpson (a) and Shannon (b) diversity is shown for a set of different hosts. Significant differences are indicated by asterisks and were calculated using the Wilcoxon rank sum test. P-values: p<0.001:'***', 0.0011>p<0.01:'**', 0.011>p<0.05:'*'. P-values are given in the Table S2.

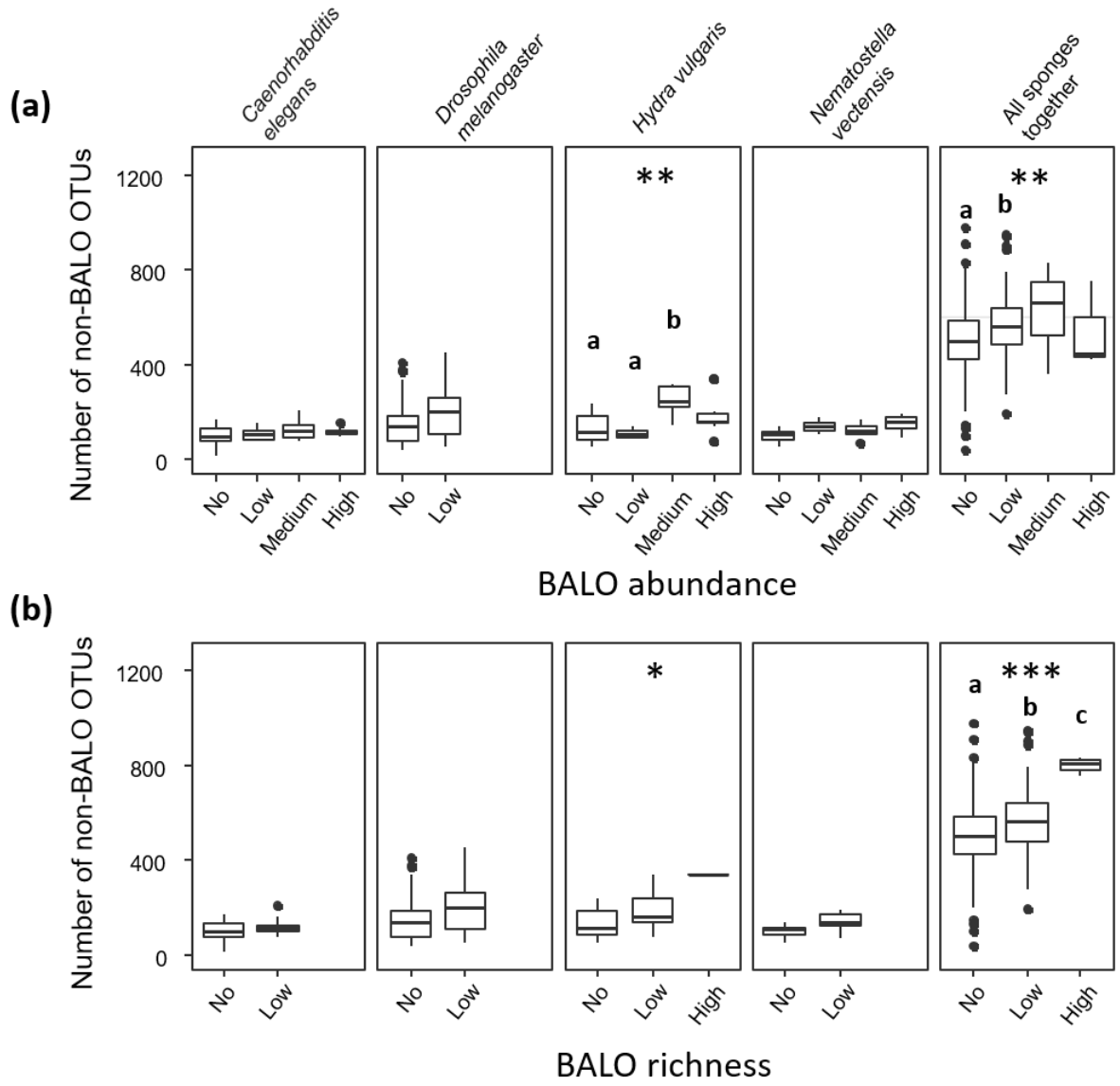

Fig. 2. Host microbiome richness measured as number of different non-BALO OTUs with increasing BALO abundance (a) and BALO richness (b). Significant differences are indicated by asterisks and were calculated using the Kruskal-Wallis rank sum test. P-values: p<0.001:'***', 0.0011>p<0.01:'**', 0.011>p<0.05:'*'. Significant differences between single categories of BALO abundance and BALO richness are indicated by different letters and were calculated with Dunn's post hoc test. All P-values are given in the Table S4.

In contrast, variation in microbiome beta-diversity was not linked to the BALOs (Figure 3). At the same time, our PCoA analysis indicated an influence of BALOs on sample clustering for several hosts (especially cnidarians and *C. elegans*). However, the clustering was not independent of sample type, making it impossible to infer the exact cause of clustering from the current data.

To exclude that BALO presence is caused by high microbiome diversity as a consequence of sampling effects, we analyzed the complete sponge dataset, additionally including species without BALOs [19]. We found that alpha-diversity *per se* does not predict the presence of BALOs (Table S5 and S6), which is therefore unlikely caused by sampling effects alone.

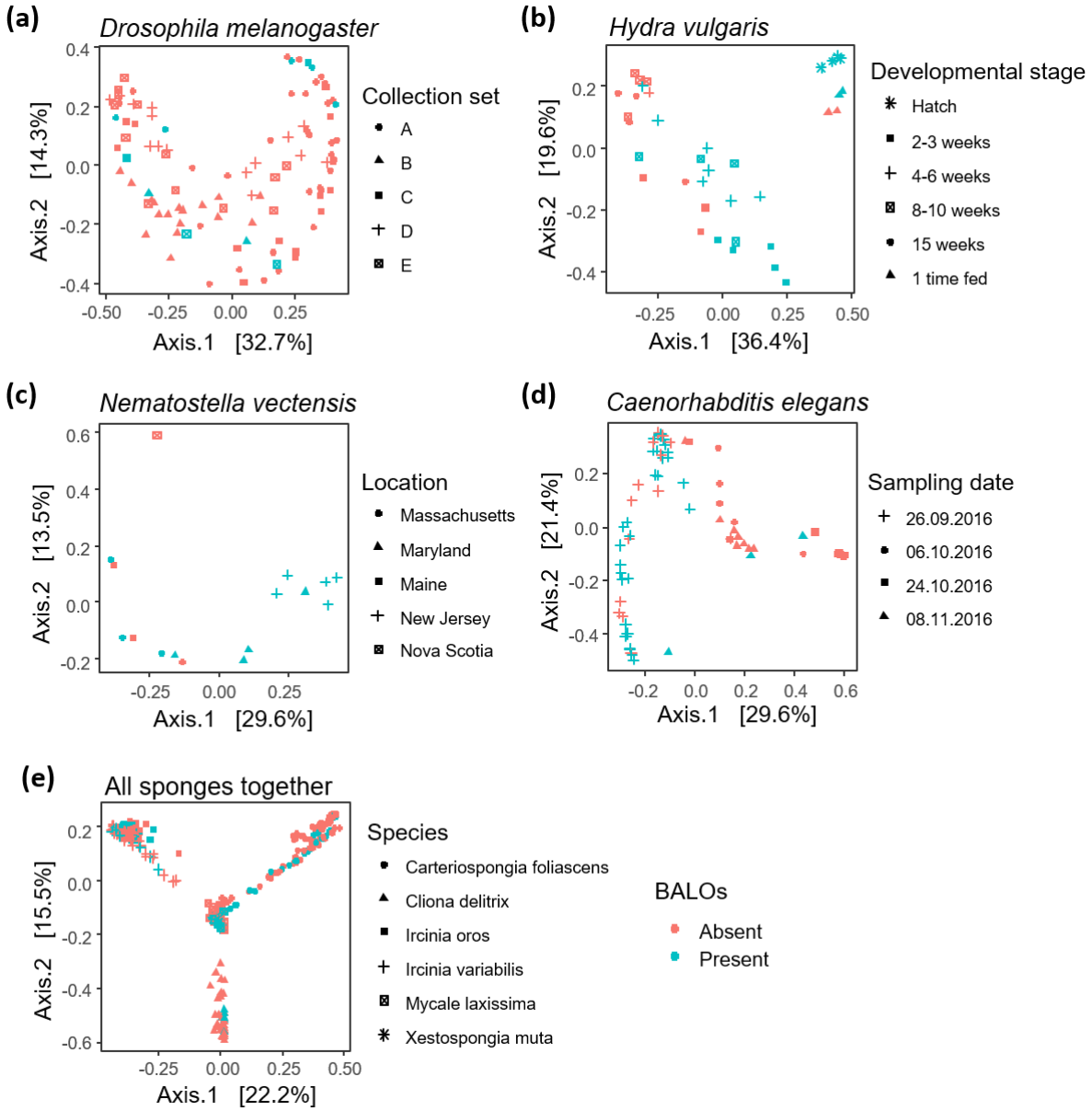

Fig. 3. PCoA of microbiome samples from different hosts using Bray Curtis distances. Samples are color-coded by presence and absence of BALOs. Different shapes indicate different sample subsets as indicated by the respective legends.

The loss of top predators has comprehensive effects on community structure [9, 10]. We tested this idea by comparing microbiome alpha-diversities for distinct animal hosts and environments that either lacked or contained a prominent group of microbial predators, the BALOs. With the exception of the considered insects and most sponge species, we found that microbiomes containing BALOs were characterized by a significantly higher alpha-diversity.

In contrast to the overall results, two sponge species showed a negative correlation between BALO presence and microbiome diversity, although not when considering microbiome richness. These results may suggest that BALO-containing sponges harbor a more species-rich, but less even microbiome. Notably, sponges in general

possess a comparatively species-rich microbiome (Figure S1). In these cases, evenness may be negatively correlated to richness, consistent with previous observations for plant communities [20] and possibly due to sampling effects, where a superior competitor is more likely present in species-rich communities [1]. A niche-preemption model was previously identified to be the best predictor for the patterns in plant communities [20]. Niche-preemption should favor resource use plasticity among the less competitive species, resulting in lower growth and consequently reduced evenness. In case of the sponges, the negative richness-evenness-relationship might then overshadow the effect of BALOs on microbiome diversity. Temporal effects could additionally explain the higher sponge microbiome diversity in the absence of BALOs. As the sponge data used in this study came from single time point samples, we cannot exclude subsequent changes in the community structure, for example a delayed effect of BALO loss or gain on microbiome diversity. However, the longitudinal data on surface microbiomes [21] indicates that changes in BALO presence/absence are associated with more or less simultaneously occurring changes in OTU richness (Fig. S2).

We found that BALO OTU richness, rather than abundance, is significantly associated with microbiome richness in *H. vulgaris* and the combined set of sponges. Moreover, this significant association between BALO and microbiome richness was only observed when the high BALO richness category could be included. Considering that different BALO strains are known to vary in their range of suitable prey [22], the above results may suggest that a more diverse BALO community is able to prey on a more diverse set of bacteria and thereby reduces the predation pressure on single species, thus increasing microbiome diversity.

Our additional analysis of beta-diversity did not reveal a strong BALO influence on microbiome community structure. Together with the results on alpha-diversity, this may imply that BALO presence is not correlated with a specific community composition and that BALOs survive in a range of differently assembled communities.

Our results from a range of distinct animal hosts and environments point to BALOs as potential drivers of microbiome alpha-diversity, possibly by actively preying on highly abundant species, thereby favoring rare species. Thus, BALOs may be of particular importance for our understanding of the stability and resilience of microbiome ecosystem functions. Our current meta-analysis is, however, based on associations, which can only be indicative of possible causal relationships. An important next step should therefore be a detailed experimental analysis of the exact causal role of BALOs on microbiome diversity and resulting functions. It would be of similar high interest to assess to what extent other kinds of bacterial antagonists, such as phages, or environmental stressors may also influence microbiome diversity and the associated effects. Moreover, it is worth testing whether the interaction between BALOs and other bacteria is additionally shaped by the host immune system, which could cause different dynamics of the BALO-mediated effects within rather than outside host organisms.

Considering that BALOs are not pathogenic to higher organisms [23], have a likely stronger effect on community structure than phages [18], and appear to enhance microbial diversity, they are highly promising candidates for probiotic therapy [24] that aims at restoring disturbed microbiomes and improving host health or ecosystem productivity and stability.

Acknowledgement

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Conflict of interest statement

The authors declare no conflict of interest.

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Supplementary Material

Bdellovibrio and Like Organisms are Predictors of Microbiome Diversity across Diverse Host Groups

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1 Supplementary Methods

For our analysis, we randomly selected exemplary host taxa that are representative of distinct taxonomic animal groups, ranging from very simple to more complex hosts and including early branching invertebrates, ecdysozoa, and also vertebrates (Table S1). In addition, we only considered host taxa, for which a single study included at least five samples with and without BALOs - with the exception of the *Nematostella* dataset with only four samples without BALOs. This preselection was performed in order to allow a direct comparison of samples with and without BALOs for each host system or environment. Further, only studies with publicly available OTU tables were selected. Moreover, we considered one study with longitudinal data generated from human mucus, sebum, skin swabs, as well as from different surfaces from their family homes [10]. This data set served to test the stability of the association of BALO presence and bacterial community diversity across time within the same environment.

Several datasets were from microbiomeDB (http://microbiomedb.org/mbio/) and only included relative abundance data, while the remaining data sets also had information on absolute frequencies. The *Caenorhabditis elegans* dataset was produced by us for this study by sampling worms from the Kiel Botanical garden in 2016 at four consecutive time points (one in October, two in September, and one in November). Worm samples were prepared as described previously [1] by using the protocol for “natural worm” microbiome extraction. DNA was sequenced using the Miseq platform and the primers 515f-806r to sequence the V4 region of the 16S rRNA gene. Original sequence data are available from the European Nucleotide Archive (accession number PRJEB30476). Sequence reads were analyzed using Mothur v. 1.39.5 [2] as described in the Miseq SOP (https://www.mothur.org/wiki/MiSeq_SOP) and the SILVA reference database version 128. OTU clustering was based on 97% sequence identity. Samples with BALOs were categorized based on their abundance (i.e., high (11-227 reads), medium (6-10), low (1-5), and no reads) and richness (high (5-7), low (1-4), and no). We compared microbiome alpha-diversity in the presence and absence of BALOs using two-sample Wilcoxon rank sum tests to account for outliers. We assessed the influence of BALO abundance or richness on microbiome richness with the Kruskal-Wallis rank sum test and Dunn’s post hoc test with p-value adjustment using fdr. Beta-diversity was measured using Bray Curtis distance on relative abundance and visualized using PCoA of the 500 most abundant OTUs. Sponge samples were analyzed using Fisher’s exact test and the Wilcoxon rank sum test to test for an association between BALO presence/absence and microbiome alpha-diversity, either as categorical or continues variable. All statistical analyses were performed in R [3] using phyloseq [4] and vegan [5].

339 ## 2 Supplementary Figures and Tables

340 Table S1: Summary of the considered and analyzed studies.

Study	Host body site	Seq. platform	Environment of host	N samples	Normalization	Further information
			***Caenorhabditis elegans* (Nematoda)**			
This study*	Gut	Miseq (V4)	Natural isolates from compost heaps	73	4986 reads per sample	Time series
			***Nematostella vectensis* (Cnidaria)**			
[6]	Whole animals	454 (V2)	Natural isolates, but maintained in the lab for 10 years as clonal lines	16	3000 reads per sample	Microbiome diversity of species sampled along the US east coast
			Six sponge species			
[7]	Random sponge pieces	Hiseq (V4)	Natural isolates from different sites	315 samples	No normalization	Different species and different sampling sites
			***Drosophila melanogaster* (Insecta, Diptera)**			
[8]	Whole flies	Miseq (V3-V4)	Samples taken from various kitchen	79	1200 reads per sample	Only adult flies
			***Apis mellifera* (Insecta, Hymenoptera)**			
Unpublished, Dominguez-Bello	whole head, whole larva, whole pupa, whole gut	Unknown	Unknown	383	Unknown	From microbiomeDB, different functional guilds and developmental stages, effect of Tetracycline application
			***Canis lupus familaris* (Vertebrata, Mammalia)**			
[9]	Sebum	Illumina GAIIx (V2)	Different homes	145	5000 reads per sample	From microbiomeDB, most BALOs in sebum und mucus
			Home surfaces			
[10]	Different surfaces from family homes	Hiseq (V4)	Different homes	690	2500 reads per sample	From microbiomeDB
			***Homo sapiens* (Vertebrata, Mammalia)**			
[10]	Mucus, sebum, skin swabs	Hiseq (V4)	Different homes	910	2500 reads per sample	From microbiomeDB
			***Hydra vulgaris* (Cnidaria)**			
[11]	Whole animals	454 (V1-V2)	Lab-kept animals	36	No normalization	Developmental data

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343 Table S2: Test statistics of the comparison of microbiome alpha-diversity in the absence and
344 presence of BALOs as shown in Fig. 1.

diversity measure	host	W	P
Simpson	*Apis mellifera*	594	0.1541
Simpson	*Caenorhabditis elegans*	774	0.001617
Simpson	*Canis lupus familaris*	1382	< 0.001
Simpson	*Drosophila melanogaster*	506	0.9089
Simpson	*Homo sapiens*	57463	< 0.001
Simpson	*Nematostella vectensis*	736	0.03724
Simpson	Family homes	27095	< 0.001
Simpson	*Hydra vulgaris*	68	< 0.001
Simpson	*Carteriospongia foliascens*_Green Island	91	0.589
Simpson	*Carteriospongia foliascens*_Kimberley, Western Australia	48	< 0.001
Simpson	*Carteriospongia foliascens*_Orpheus Island, Little Pioneer Bay	50	< 0.001
Simpson	*Cliona delitrix*_Caribbean	140	0.3135
Simpson	*Ircinia oros*_Spain	148	0.01725
Simpson	*Ircinia variabilis*_Spain	425	< 0.001
Simpson	*Mycale laxissima*_Enrique Cay, Puerto Rico	35	0.1653
Simpson	*Xestospongia muta*_Boynton Beach, FL	5	0.04798
Simpson	All together	486490	< 0.001
Shannon	*Apis mellifera*	557	0.1151
Shannon	*Caenorhabditis elegans*	792	0.0025
Shannon	*Canis lupus familaris*	1019	< 0.001
Shannon	*Drosophila melanogaster*	487	0.9348
Shannon	*Homo sapiens*	49350	< 0.001
Shannon	*Nematostella vectensis*	729	0.0327
Shannon	Family homes	22200	< 0.001
Shannon	*Hydra vulgaris*	58	< 0.001
Shannon	*Carteriospongia foliascens*_Green Island	85	0.4231
Shannon	*Carteriospongia foliascens*_Kimberley, Western Australia	45	0.004662
Shannon	*Carteriospongia foliascens*_Orpheus Island, Little Pioneer Bay	41	0.05528
Shannon	*Cliona delitrix*_Caribbean	159	0.6025
Shannon	*Ircinia oros*_Spain	185	0.1247
Shannon	*Ircinia variabilis*_Spain	417	< 0.001
Shannon	*Mycale laxissima*_Enrique Cay, Puerto Rico	35	0.1653
Shannon	*Xestospongia muta*_Boynton Beach, FL	6	0.07323
Shannon	All together	415580	< 0.001

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346 Table S3: Test statistics of the comparison of microbiome richness in the presence and
347 absence of BALOs as shown in Fig. S1.

Host	W	P
Caenorhabditis elegans	178.5	0.06802
Drosophila melanogaster	348	0.1102
Hydra vulgaris	88.5	0.003041
Nematostella vectensis	11	0.1293
All sponges together	4531.5	< 0.001
***Carteriospongia foliascens*_Green Island**	144	< 0.001
***Carteriospongia foliascens*_Kimberley, Western Australia**	70	0.9321
***Carteriospongia foliascens*_Orpheus Island, Little Pioneer Bay**	116	0.4946
***Cliona delitrix*_Caribbean**	468	0.021
***Ircinia oros*_Spain**	518	< 0.001
***Ircinia variabilis*_Spain**	664	0.01112
***Mycale laxissima*_Enrique Cay, Puerto Rico**	220	0.9319
***Xestospongia muta*_Boynton Beach, FL'**	42	0.1058

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Table S4: Test statistics of the comparison of microbiome richness and BALO abundance and BALO richness as shown in Fig. 2.

host	category	Kruskal-Wallis Chi-squared	Df	P	Significant Dunn's
Caenorhabditis elegans	BALO abundance	3.3508	1	0.06717	
Drosophila melanogaster	BALO abundance	6.3712	3	0.09488	
Hydra vulgaris	BALO abundance	12.795	3	0.005102	Medium:Low P = 0.014, No:Medium P = 0.011
Nematostella vectensis	BALO abundance	3.7776	3	0.2865	
All sponges together	BALO abundance	14.573	3	0.002221	No:Low P = 0.0033
Caenorhabditis elegans	BALO diversity	4.2926	3	0.2316	
Drosophila melanogaster	BALO diversity	2.5684	1	0.109	
Hydra vulgaris	BALO diversity	6.7652	2	0.03396	
Nematostella vectensis	BALO diversity	2.489	1	0.1146	
All sponges together	BALO diversity	17.87	2	< 0.001	No:Low P = 0.0032, No:High P = 0.0053, Low:High P = 0.0349

Table S5: Test statistics of the comparison of sponge microbiome alpha-diversity category and BALO presence. Contingency tables are based on the average of the respective value (given in brackets for the different categories) for each species.

	Microbiome richness[a]		**Fisher's Exact Test**	
	high (>=600 - 809.25)	low (306.67 - <600)	P	Odds ratio
BALOs present	10	38	0.7356	0.79247
BALOs absent	4	12		

	Microbiome Simpson diversity		**Fisher's Exact Test**	
	high (>=0.9)	low (0.38 - <0.9)	P	Odds ratio
BALOs present	17	31	1	1.20297
BALOs absent	5	11		

	Microbiome Shannon diversity		**Fisher's Exact Test**	
	high (3.5 - 4.74)	low (1.7 - <3.5)	P	Odds ratio
BALOs present	23	25	1	1.17976
BALOs absent	7	9		

[a] Microbiome richness is treated as a categorical variable, being either high or low. Cut-offs for the two groups are indicated.

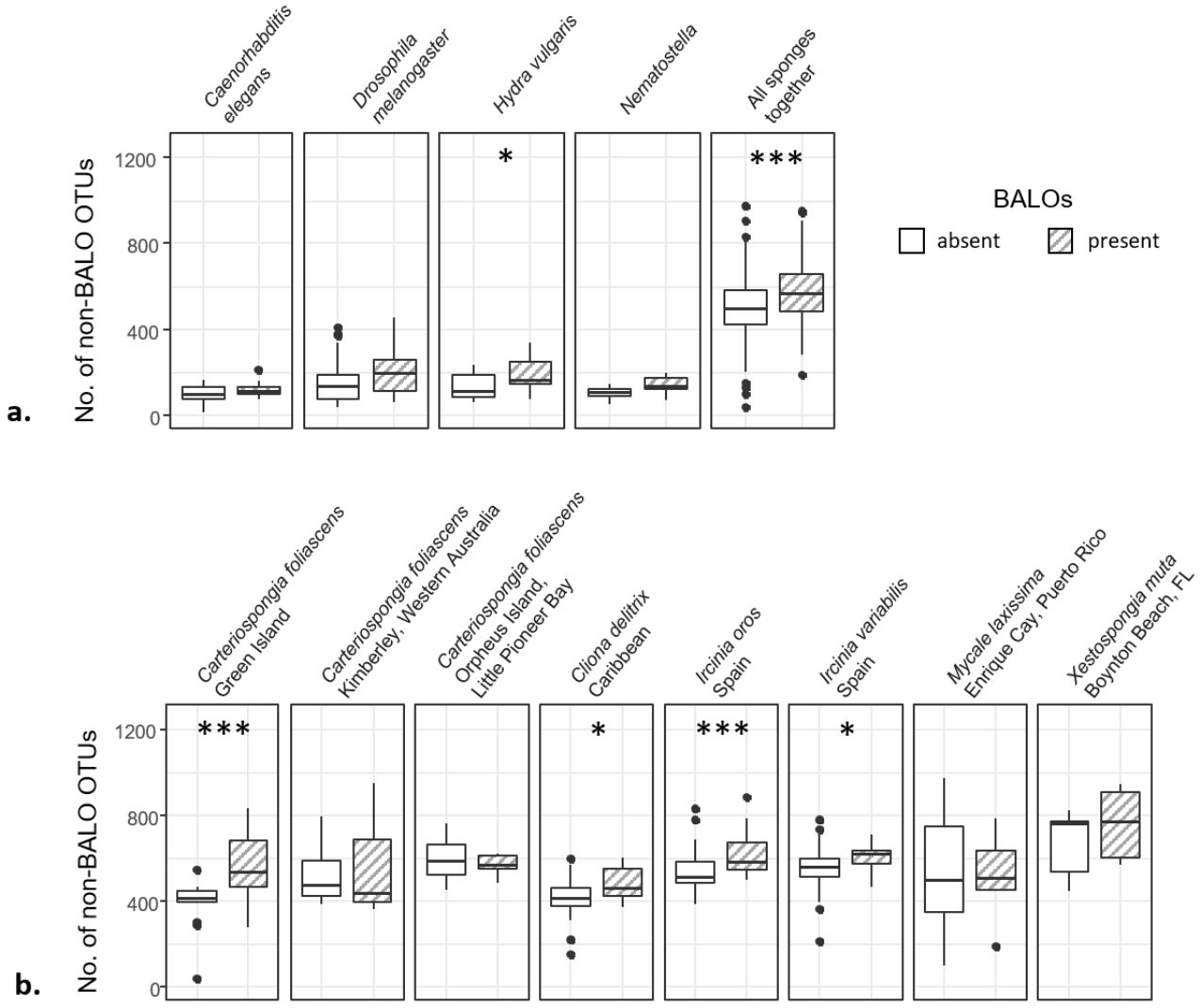
358 Table S6: Wilcoxon rank sum test statistics of the comparison of sponge microbiome alpha-
359 diversity in samples either with BALO presence *versus* BALO absence.

Diversity measure[a]	W	P
Simpson	324	0.3598
Shannon	329	0.4018
OTU richness	364	0.7647

360 [a] Microbiome diversity is used as a continuous variable and compared among the two
361 groups, which are either defined by the presence or the absence of BALOs.

362

363



364

365 Fig. S1: Microbiome richness of different hosts (a) and particular sponge species (b)
366 measured as number of different non-BALO OTUs in the presence and absence of BALOs.
367 Significant differences are indicated by asterisks and were calculated using the Wilcoxon
368 rank sum test. P-values: p<0.001:'***', 0.0011>p<0.01:'**', 0.011>p<0.05:'*'. P-values are
369 given in the Table S3.

370

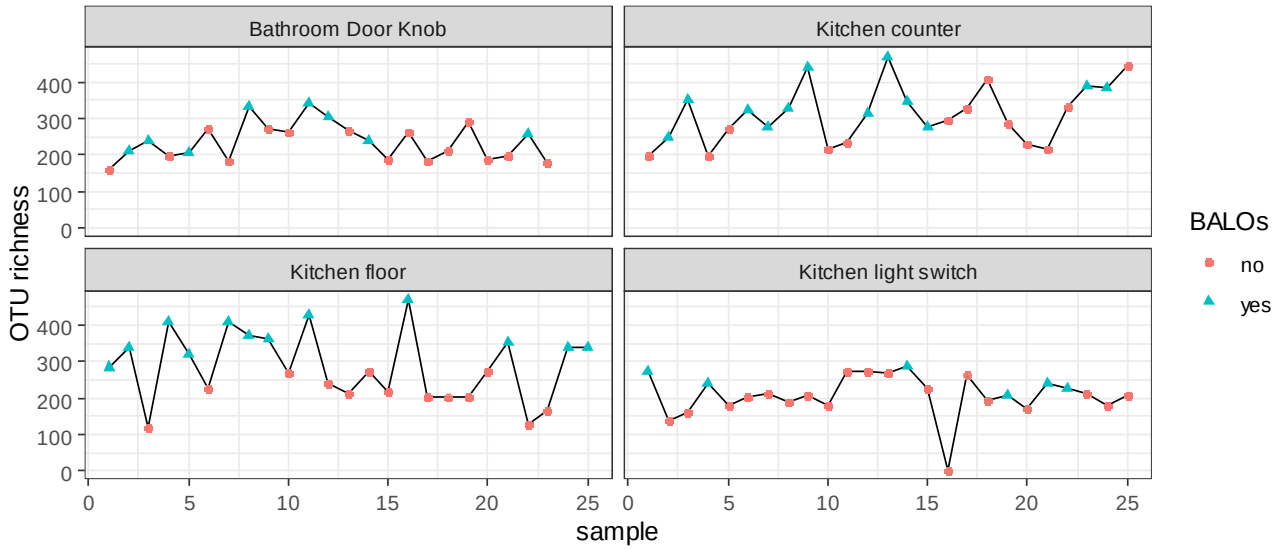


Fig. S2: Microbiome richness of longitudinal samples from house 05b from [10]. Samples were taken every other day. Data points are colored and shaped according to the presence or absence of BALOs.

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